single-molecule measurements and physiological forces. We believe that our work provides first steps towards a theoretical framework to better understand dynamic and cellular protein biomechanics and biological force generation.
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1124-Plat
Probing Protein Folding Kinetics with High-resolution, Stabilized Optical Tweezers
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Single-molecule techniques provide a powerful means of exploring molecular transitions such as the unfolding and refolding of a protein. However, the quantification of bi-directional transitions and near-equilibrium phenomena poses unique challenges, and is often limited by the detection resolution and long-term stability of the instrument. We have developed unique optical tweezers methods that address these problems, including an interference-based method for high-resolution 3D bead tracking (~1 nm laterally, ~0.3 nm vertically, at > 100 Hz), and a continuous autofocus system that stabilizes the trap height to within 1-2 nm longterm [1-3]. We have used our instruments to quantify the force-dependent unfolding and refolding kinetics of single protein domains (e.g. spectrin [3,4]). These single-molecule studies are presented, together with the accompanying probabilistic analysis that we have developed.
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Platform W: Anyloids from Multiple Perspectives

1125-Plat
Possible Mechanism Of Amyloid Formation By Apomyoglobin Mutants
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It is known that even proteins, not involved into diseases, are able to form amyloid-like structures similar in final architecture of their fibrils. This fact suggests that formation of aggregated cross-beta structure is a common property of a polypeptide chain under appropriate conditions. Sperm whale apomyoglobin was used to investigate amyloid formation because the properties and folding process of this protein are well known. Process of the apomyoglobin mutant aggregation was monitored under conditions close to physiological ones (40°C, pH 5.5) by ThT binding, turbidity, FTIR spectroscopy and electron microscopy. Mutated proteins contained a single point substitution at positions Val10 and Met131 by Ala, Phe and Trp. It was shown that the WT apomyoglobin formed aggregates not containing beta-structure, while variants of apomyoglobin have shown significant increase of ThT fluorescence intensity and changes in a form of FTIR spectra. These changes evidenced appearance of beta-structured aggregates, and EM images showed fibril-like aggregates. Kinetics of amyloid formation monitored by turbidity and ThT binding allowed to calculate three rate constants of amyloid formation and to distinguish three stages of this process. Obtained results suggest that the rate of the first stage is affected by a position of substitution, and is not influenced by its type. In contrast, the rate of the second stage depends on a type of substitution: it is slower for mutants with aromatic amino acid substitutions. This work was supported by INTAS grant 05-1000004-7747, partly by the Howard Hughes Medical Institute Award 55005607 to A.V. Finkelstein and by the RAS Program on ''Molecular and Cellular Biology''.

1126-Plat
Investigations of Amyloid Fiber Formation of Alpha-Synuclein and Amyloid-beta Using Newly Synthesized Small Molecules
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Alpha-Synuclein and Amyloid-beta are amyloid forming proteins which aggregate in Parkinson's and Alzheimer's disease brain, respectively. We designed and synthesized a novel boronic acid- and chromene-based small molecule library, and tested the molecules' *in vitro* activity against alpha-Synuclein and Amyloid-beta by examining the effect on the aggregation process. The aggregation was monitored using the amyloid-specific Thioflavin T fluorescence, as well as by native gel electrophoresis, and transmission electron microscopy. We observed that some compounds were effective at stabilizing the initial species, while others appear to stabilize a ring-like oligomeric intermediate as observed by electron microscopy combined with single particle analysis. Furthermore, some compounds were able to promote the formation of amyloid fibers. Together, these results serve as a foundation for the future design of small molecule inhibitors and diagnostic agents (PET-agents) for amyloid fibers. In addition, they provide insights into the mechanism of aggregation in many neurodegenerative diseases.

1127-Plat
Using Pressure Perturbation for Studying the Free Energy and Conformational Landscape of Proteins Upon Aggregation and Amyloid Formation
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Pressure tuning in combination with calorimetric, spectroscopic and structural techniques (DSC, PPC, FTIR, SAXS, AFM) revealed new insights into the pre-aggregated regime as well as mechanistic details about concurrent aggregation pathways and the differential stability of insulin aggregates. A thorough thermodynamic approach has provided a coherent and precise description of changes of the partial specific volume, heat capacity, the coefficient of thermal expansion, as well as the adiabatic and isothermal compressibility of the protein upon unfolding and aggregation. This was only possible due to a novel application of ultrasound velocimetry and pressure perturbation calorimetry. Besides pressure, also solvational perturbations, accomplished by the addition of various salts and cosolvents such as glycerol, ethanol and TFE, have been explored. They exert pronounced and diversified effects on the unfolding, non-native assembly and fibril formation, which ultimately manifest in morphological variations of mature aggregates and fibrils (strains). The phenomenon of strains easily fits to a generalized protein energy landscape picture involving an alternative comb-shaped aggregation funnel. The pressure variable has also been explored to study more disease related amyloidogenic proteins, such as PrP and IAPP. Several examples will be given.

1128-Plat
Amyloid Peptide Aggregation In Plugs Formed By Microfluidics
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We present a novel microfluidic device for amyloid peptide aggregation research. The device relies on the control of interfacial chemistry, which allows miniaturizing of aggregation measurements to nanoliter volumes. In traditional in vitro aggregation experiments, adsorption of amyloid peptides to various interfaces has been shown to nucleate and to enhance peptide aggregation. The problem of adsorption is even more pronounced upon miniaturization of aggregation experiments. Miniaturization leads to an increase of the surface-to-volume ratio, and concomitantly to an increase of amyloid peptide aggregation if the surface is not controlled. Nevertheless, miniaturization of aggregation experiments is desirable for samples available only in small volumes, as for example cerebrospinal fluid (CSF) from mice. CSF has recently gained interest in Alzheimer research, however CSF analytics has been hampered to due the small available volume.
In order to miniaturize and control the interfacial chemistry of aggregation experiments we used a plug based microfluidic approach. Plugs are nanoliter sized aqueous droplets formed in the flow of immiscible fluids inside microfluidic channels. Upon peptide encapsulation into plugs, the unfavorable interfaces are exchanged for an adjustable liquid/liquid interface. We show for one prominent amyloid peptide, the Alzheimer's peptide Aβ(1-40), that aggregation in plugs has kinetics of orders of magnitude slower than under standard conditions. Further we show the applicability of this miniaturized system to aggregation experiments by testing the inhibitory potency of CSF from wild type and ceAPPswePS1ΔE9/TTR-/- mice on Aβ aggregation. Using the plug-based approach, we were able to perform over 750 experiments with a single mouse CSF sample of 5 μl in volume. The plug system offers many new opportunities to investigate in vitro aggregation studies, as for example time controlled aging of amyloid peptides, nucleation in a confined environment, and screening of drug components.

1129-Plat
Unraveling the Polyglutamine Aggregation Pathway in Huntington's Disease by Small-Angle Neutron Scattering
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